EFFECT OF GENOTYPE ON LEVEL OF NATURAL INFECTION OF STRONGYLES IN LAMBS

I Mirza, R M Gatenby, A J Wilson, G Adiwinata and A Batubara

Working paper No. 159, June 1994

SR-CRSP, SBPT, P O Box 1, Galang 20585, North Sumatra, Indonesia

SR-CRSP, University of California, Davis, CA 95616, USA

PENGARUH GENOTYPE DOMBA TERHADAP TINGKAT INFEKSI ALAMI STRONGYLE

I Mirza, R M Gatenby, A J Wilson, G Adiwinata dan A Batubara

ABSTRAK

Suatu penelitian telah dilakukan untuk mempelajari perolehan infeksi nematoda di bawah kondisi alami dalam sekelompok domba dengan 5 genotype yang berbeda. Domba yang digunakan berjenis kelamin jantan yang berumur lebih kurang 4 bulan.

Pada permulaan penelitian semua domba diberikan obat cacing, setelah itu digembalakan pada lahan perkebunan karet selama 1 bulan. Kemudian domba dikurung selama 1 bulan lagi agar nematoda yang sudah terinfeksi mencapai pendewasaan. Pada akhir penelitian, semua domba dipotong dan traktus gastrointestinal dibersihkan yang selanjutnya dilakukan penghitungan dan identifikasi cacing.

Penelitian ini mempunyai 3 ulangan dalam season yang berbeda. Pada masing-masing ulangan digunakan 4 ekor domba. Genotype yang diuji pada penelitian ini adalah lokal Sumatera ekor tipis, silangan Sumatera dan Barbados Blackbelly, silangan Sumatera dan ekor gemuk, silangan Sumatera dan St Croix (Virgin Island) turunan pertama, dan silangan Sumatera dan St Croix turunan kedua.

Parameter yang diukur adalah berat badan, faecal egg counts dan jumlah cacing. Faecal egg counts dan jumlah cacing merupakan indikasi infeksi cacing yang diperoleh selama 1 bulan periode penggembalaan.

Haemonchus contortus dan Cooperia spp. merupakan nematoda yang paling umum dijumpai. Trichostrongylus spp., Oesophagostomum spp. dan Strongyloides papillosus dijumpai dalam jumlah sedikit. Eurytrema pancreaticum juga umum dijumpai khususnya pada ulangan kedua.

Perbedaan diantara genotype dijumpai pada faecal egg counts di 2 ulangan ($P<0.01$), silangan Barbados Blackbelly dan turunan kedua St Croix mempunyai egg counts yang rendah. Bagaimanapun tidak banyak dijumpai perbedaan antara genotype pada jumlah cacing pada setiap ulangan.

EFFECT OF GENOTYPE ON LEVEL OF NATURAL INFECTION OF STRONGYLES IN LAMBS

I Mirza, R M Gatenby, A J Wilson, G Adiwinata and A Batubara

ABSTRACT

An experiment was conducted to study the acquisition of nematode infection under natural conditions in groups of lambs belonging to 5 different genotypes. Lambs aged about 4 months were used.

At the start of the experiment the lambs were drenched, after which they were allowed to graze in a rubber plantation for one month. Thereafter they were penned for another month to allow maturation of acquired nematode infection. At the end of the experiment, they were all slaughtered and their complete gastrointestinal tracts removed for subsequent worm counts and identification.

The experiment had three replicates, in different seasons. Four animals per group were used in each replicate. The genotypes tested in this study were, local Sumatra Thin-tail, crosses of Sumatra and Barbados Blackbelly, Sumatra and Javanese fat-tail, Sumatra and St Croix (Virgin island) in their first generation, and Sumatra and St Croix in their second generation.

Parameters studied were body weight, faecal egg counts and total worm counts. Both faecal egg counts and worm counts indicated that substantial worm infections were acquired during the one month grazing period.

Haemonchus contortus and Cooperia spp. were the most common nematodes encountered. Trichostrongylus spp., Oesophagostomum spp. and Strongyloides papillosus occurred in low numbers. Eurytrema pancreaticum was also common, especially in the second replication.

Differences between genotypes were found in the faecal egg counts in 2 replications ($P<0.01$), the Barbados Blackbelly crosses and the second generation of St Croix crosses having the lowest egg counts. However,no substantial differences between genotypes were found in the worm counts in any of the replications.

INTRODUCTION

Rubber plantations, which cover a wide area of North Sumatra offer great potential for sheep production. The undergrowth in plantations has been found suitable for grazing sheep and in turn the grazing of these animals may reduce expenditure on weedicides. Unfortunately the moist conditions prevailing under rubber are very favourable for the development and survival of the pre-parasitic stages of strongyles. Control of parasites is difficult because of year-round rainfall and high temperatures.

Suppressive anthelmintic treatments are currently the only way of reducing infections but they are costly and frequent use of the same drugs may lead to the development of resistance of the worms to these medicines.

An alternative way to control parasites would be to select for breeds or strains of sheep which are more resistant to infection. Heritability of host resistance to Haemonchus contortus, the most important strongyle species in sheep, has been estimated at 0.29 (Albers et al., 1984).

The present experiment was designed to study possible differences in susceptibility to infection with internal parasites of several genotypes of sheep present at the Sungei Putih station.

MATERIALS AND METHODS

Animals and Management

The study was divided into 3 replications of which each was performed in a different season. The first replication was conducted from August to October 1992, the second replication from November 1992 to January 1993, and the third replication from February to April 1993.
The total rainfall, and mean minimum and maximum temperatures during the first part of the experiment (grazing) are summarised in Table 1.

Table 1. The total rainfall (mm) and mean minimum and maximum temperatures (^{0}C) when the animals were grazing.

	Temperature (^{0}C)		Rainfall(mm)
	Min	Max	
Replication 1 August 1992	22.5	32.0	150
Replication 2 November 1992	22.4	30.4	144
Replication 3 February 1993	21.7	31.2	34

In each replication 16 lambs were used. They were divided into 4 groups of 4 animals each belonging to different genotypes.
In replication 1, genotypes S, B1, E1 and H1 were included; in replication 2 and 3, there were genotypes S, E1, H1 and HC.

1. Local Sumatra	S
2. Barbados Blackbelly x Sumatra; first generation	B1
3. Java Fat-tail x Sumatra; first generation	E1
4. Virgin Island (St Croix) x Sumatra; first generation	H1
5. Virgin Island (St Croix) x Sumatra; second generation	HC

At the start of each experiment, all lambs were given Febantel (Rintal, Bayer) orally at a dose of 10 mg/ kg body weight, after which they were allowed to graze in a rubber plantation which was known to be contaminated with strongyles.
Grazing time was fixed at 4 h/day for a period of 4 weeks. Thereafter all animals were kept indoors for another 4 weeks and fed with concentrate and fresh grass cut from an uncontaminated pasture.

At the end of this period all animals were slaughtered and their complete gastrointestinal tracts removed for subsequent worm counts and identification.

Measurements

All lambs were weighed and faecal sampled at weekly intervals until slaughter. Strongyle egg counts were performed on the faecal samples, using a modified McMaster technique with a sensitivity of 30 eggs per gram faeces (EPG) for each egg counted.
Worms found in the abomasum, small intestine, large intestine and pancreas, were identified and counted using standard techniques.

The pancreas was separated from the gastrointestinal tract and weighed on an electronic scale.

Data analysis

The data of faecal egg counts (EPG) were transformed for statistical analysis, using the standard transformation log(EPG+1). Geometric mean egg counts were calculated by exponential reconversion.

The data of body weights, faecal egg counts (of the 5 last sampling dates only) the numbers of nematodes, and Eurytrema pancreaticum and the pancreas weight were statistically analysed using the analysis of variance procedure and Duncan's multiple range test.

RESULTS AND DISCUSSION

Growth rate

The mean daily growth rates (g/d) for each genotype in the 3 replication are presented in Table 2. They were calculated for each animal from the body weight, recorded weekly, against time.

The overall mean daily growth rate was 71 g/d. No statistically significant effect of genotype on weight gain was found in any of the replications.

Table 2. Growth rates (g/d) for each replication

	Replication 1	Replication 2	Replication 3
Breed Type	Mean±SE	Mean±SE	Mean±SE
B1	109±11.0	- -	- -
E1	77± 5.5	62±11.0	52±10.0
H1	93±12.5	67±13.5	72± 8.0
S	77±10.5	74±15.0	51±15.5
HC	- -	61±12.5	58± 5.0
Significance of difference	ns	ns	ns

ns : not significant

Strongyle faecal egg counts

Overall mean transformed strongyle egg count (EPG) was 3.64 which corresponds to a geometric mean of 4410 EPG. A significant ($P<0.01$) effect of genotype on strongyle faecal egg counts was found in replications 1 and 2, but not in replication 3. In replication 1, the mean EPG of breed type B1 was significantly lower than that of the other breed types tested, namely E1, H1 and S. E1 lambs had the highest mean EPG in replication 1 and 3, and the second highest EPG in replication 2. There were no consistent effects of H1 and HC compared to S lambs.

Table 3. Nematode faecal egg counts

Breed Type	Replication 1		Replication 2		Replication 3	
	Log(EPG+1) Mean ± SE	Geo. mean EPG	Log(EPG+1) Mean ± SE	Geo. mean EPG	Log (EPG) Mean ± SE	Geo. mean EPG
B1	3.40^{a} ±0.11	2510	-		-	
E1	3.60^{c} ±0.11	3980	3.75^{bc} ±0.07	5620	3.91±0.10	7940
H1	3.58^{bc} ±0.12	3800	3.79^{c} ±0.08	6170	3.76±0.11	5750
S	3.57^{b} ±0.15	3710	3.46^{ab} ±0.09	2880	3.83±0.06	6760
HC	-	-	3.30^{a} ±0.12	1990	3.80±0.08	6310
Significance of difference	P<0.01		P<0.01		ns	

Means with different superscripts are significantly different ($P<0.01$)

Nematode counts

The mean number of worms recovered from the gastrointestinal tracts are summarised in Table 4. *Haemonchus contortus*, *Cooperia* spp. and *Trichostrongylus* spp. were the most common nematodes encountered. *Oesophagostomum* spp. and *Strongyloides papillosus* were present in low numbers. Significant differences between genotypes were only found for *S. papillosus* in replication 1 and *Oesophagostomum* spp. in replication 2.

Table 4. Number of nematodes in abomasum, small and large intestine. Mean±SE (Standard Error)

Breed Type	Haemonc.	Coop.	Tricho.	Oesoph.	Strongy.
Replication 1	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
B1	721±266.5	124±47.5	58± 8.0	0.25±0.25	4±2.5
E1	873± 39.5	131±54.5	73±54.5	0.25±0.25	1±1.5
H1	810±151.0	164±71.0	69±26.5	2.00±1.69	11±4.5
S	961±193.0	174±65.5	95±44.0	0.00±0.00	0±0.0
Significance of difference	ns	ns	ns	ns	P<0.05
Replication 2					
E1	719± 56.5	294±40.0	19±6.5	1.0±0.00	0±0
H1	810±467.0	250±123.0	23±8.0	1.0±0.40	0±0
S	329±112.5	188± 52.5	11±6.5	1.5±0.85	0±0
HC	498±161.5	309± 93.5	65±20.5	0.5±0.50	0±0
Significance of difference	ns	ns	ns	P<0.05	ns
Replication 3					
E1	534±72.5	965±369.5	270±64.0	41±14.5	0±0
H1	448±44.0	1045±208.0	144±25.5	38± 7.5	0±0
S	501±97.0	931±134.0	200±39.0	24± 6.0	0±0
HC	390±76.5	1514±611.0	195±71.0	43± 6.5	0±0
Significance of difference	ns	ns	ns	ns	ns

Symbols: Haemonc. *Haemonchus contortus*
Coop. *Cooperia* spp.
Tricho. *Trichostrongylus* spp.
Oesoph. *Oesophagostomum* spp.
Strongy. *Strongyloides papillosus*

Haemonchus contortus

The overall mean number of Haemonchus contortus in the abomasum was 630 per animal. Except for replication 3 where there were large numbers of Cooperia, there were more Haemonchus contortus than other species of worms. There were no statistically significant differences between breed types in number of Haemonchus contortus, nor any consistent trend.

Cooperia

The overall mean number of Cooperia spp. was 510 per animal, which made this worm the second most important. Cooperia spp. even outnumbered Haemonchus contortus in the 3rd replication.

Breed type did not have a significant effect on the level of infection of Cooperia spp.

Trichostrongylus, Oesophagostomum and Strongyloides

Numbers of Trichostrongylus, Oesophagostomum and Strongyloides were low in the 3 replications.

Eurytrema pancreaticum

The prepatent period of Eurytrema pancreaticum is reported to be 80 -100 days (Soulsby, 1982). So, infections observed in this study must have partly been acquired before the start of the experiments. Results are therefore difficult to evaluate.

Infections were highest in the second replication (Table 5), the overall mean was 197 per animal. There were statistically significant differences between breed types ($P<0.01$) in this experiment.

Table 5. Number of pancreatic fluke

Breed Type	Replication 1	Replication 2	Replication 3
	Fluke	Fluke	Fluke
	Mean±SE	Mean±SE	Mean±SE
B1	68±25.5	-	-
E1	0± 0.0	18±15.0	70±57.0
H1	20±14.0	165±98.0	82±47.0
S	52±32.5	162±58.5	28±28.0
HC	-	443±83.0	35±24.0
Significance of difference	ns	P<0.01	ns

Table 6. Pancreas weight for each breed type

Breed Type	Replication 1 Panc.Wt. (g) Mean±SE	Replication 2 Panc.Wt. (g) Mean±SE	Replication 3 Panc.Wt. (g) Mean±SE
B1	-	-	-
E1	-	22.15±2.26	18.30±2.74
H1	-	24.08±3.89	20.49±1.21
S	-	22.00±2.26	20.35±0.79
HC	-	29.10±3.22	20.16±2.94
Significance of difference	-	ns	ns

GENERAL DISCUSSION

Both faecal egg counts and post-mortem worm counts indicated that substantial nematode infections were acquired in lambs during the one month grazing period. H. contortus and Cooperia spp. were the most important nematodes in terms of numbers. Large numbers of Cooperia spp. may cause symptoms of parasitic gastroenteritis and may enhance the pathogenic effects of abomasal nematodes. The intensity of infection is usually highest in non-immune young animals. H. contortus causes severe blood loss, each worm removing about 0.05 ml blood per day (Clark et al, 1962). The overall mean number of H. contortus recovered from the abomasum in the three replications was 630, which accounts for a mean daily blood loss of about 30 ml.

Infection of less than 1000 adult H. contortus will usually not cause acute clinical symptoms but may cause growth retardation, especially in young animals. The impact of the nematode infections in the present study may be evaluated by comparing the results with that of another study done at the Sungei Putih experimental farm (Mirza and Gatenby, 1993). In the present study an overall mean daily weight gain of 71 g/d was recorded with an overall mean strongyle faecal egg count of 4410; In the other experiment the weight gain was 88 g/d with a mean egg count of 24. It is probable that the lower weight gain in the present study was caused by a higher worm infection.

No significant differences in acquisition of strongyle infections were measured between replications. North Sumatra has an equatorial climate with little variation in temperature and year-round rainfall.

It is probable that development and survival of preparasitic stages of strongyle occur any time of the year (Beriajaya and Stevenson, 1985). Absence of seasonal variations in prevalence and intensity of nematode infections was also reported in sheep in Peninsular Malaysia which has a similar climate (Dorny et al, 1994).

Results of the 3 replications show little variation in the acquisition of gastrointestinal nematodes between the genotypes tested. This is in contrast with other studies done on the Sungei Putih experimental farm, that showed a higher resistance towards strongyle infections of Virgin Island breed types (Aron et al, 1994) and Barbados Blackbelly breed types (Endang et al, 1994).

The absence of breed differences in the present experiments might be due to the young age of the animals used. It has been shown that the genetic resistance of sheep to H. contortus results from the expression of an acquired immune response rather than of innate resistance (Gill, 1991). Lambs younger than six months of age respond poorly to infection with H. contortus, and this unresponsiveness may be immunologically mediated (Benitez-Usher et al, 1977). In infection experiments breed differences in resistance towards nematode infections are usually not declared during primary infections but become more obvious following challenge (Cabaret and Gruner, 1988).

E. pancreaticum is a fluke which occurs in the pancreatic duct of ruminants. It may cause ill-thrift, but is usually reported to be of little pathogenicity. The infection is usually diagnosed post mortem although the small, brown eggs may be found in the faeces. Land snails are the initial intermediate hosts and grasshoppers the second (Hammond and Sewell, 1990). An overall mean of 96 worms per animal was found in this study. Large variations between replications and between genotypes within the experiments were found. However these variations could not be explained. Since all animals had already grazed before experiment started and were not treated against pancreatic fluke prior to this experiment, at least part of the fluke burden was probably acquired before the study. This makes interpretation difficult.

However the results of this study indicate that considerably high infections with E. pancreaticum may be acquired in young animals.

CONCLUSION

Young animals acquire important infections with Haemonchus contortus an a relatively short period.

No breed difference in acquisition of infection were recorded in 4 months old animals.

ACKNOWLEDGEMENTS

We thank Mrs Melinda Hutauruk, Mr Immaniyanto and Mr A.M. Mursyid for analyzing the faecal samples, Mr Pungu Nababan, Mr Edy Susanto and Mr Ponijan for looking after the experimental animals and collecting faecal samples, Mr Leo P. Batubara for encouraging us to do this experiment and Dr. P. Dorny for correcting this working paper.

REFERENCES

Albers, G.A.A., Burgess, S.E., Adams, D.B., Barker, J.S.F., Le Jambre, L.F., Piper, L.R., 1984. Breeding Haemonchus contortus resistant sheep-problems and prospect. In "Immunogenetic approaches to the control of endoparasites". Ed. Dineen J.K. and Outteridge P.M., CSIRO, Division of Animal Health, pp 41-51.

Batubara, A., Mirza, I., Hutauruk, M., Gatenby, R.M. and Wilson, A.J. 1994. Monitoring of levels of gastro-intestinal nematodes at Sungai Putih. Working paper, SR-CRSP, SBPT, PO Box 1, Galang 20585, North Sumatra, Indonesia.

Benitez-Usher, C., Armour, J., Duncan, J.L., Urquhart, G.M. and Gettinby, G., 1977. A study of some factors influencing the immunization of sheep against Haemonchus contortus using attenuated larvae. Veterinary Parasitology, 3, 327-342.

Beriajaya, Stevenson, P., 1985. The effect of anthelmintic treatment on the weight gain of village sheep in West Java. In Proc. of the 3rd AAAP Anim. Sci. Cong., Seoul, Korea, 1, 519-521.

Cabaret, J., Gruner, L., 1988. Genetic variability of resistance parasites. In "3rd World congress on sheep and beef cattle breeding, Paris, 1, 577-592.

Clark, C.H., Kiesel, G.K., Goby, C.H., 1962. Measurements of blood loss caused by Haemonchus contortus infection in sheep. American Journal of Veterinary Research, 23, 977-980.

Dorny, P., Symoens, C., Jalila, A., Vercruysse, A.and Sani, R., 1994. Strongyle infections in sheep and goats under the traditional husbandry system in Peninsular Malaysia. Veterinary Parasitology, in press.

Gill, H.S., 1991. Genetic control of acquired resistance to haemonchiasis in Merino lambs. Parasite Immunology, 13, 617-628.

Hammond, J.A., Sewell, M.M.H., 1990. Diseases caused by helminths. In "Animal diseases in the tropics", 4th edition. Ed. Sewell M.M.H. and Brocklesby D.W., Bailliere Tindall, London, pp 158-159.

Mirza, I., Gatenby, R.M., 1993. Effect of time of grazing on worm uptake by sheep. Working paper 141, SR-CRSP, SBPT, PO Box 1, Galang 20585, North Sumatra, Indonesia.

Soulsby, E.J.L., 1982. Helminths, arthropods and protozoa of domesticated animals. Bailliere Tindall, London, 809 pp.